REMARKS

Reconsideration is requested.

Claims 22-27 and 29-40 are pending. Claims 37-40 have been allowed. See, page 1 of the Office Action dated July 1, 2003 (Paper No. 19). Claim 28 has been canceled without prejudice.

To the extent not obviated by the above, the Section 112, first paragraph, rejection of claims 22-36 and the rejection under 35 USC 132, stated in paragraphs 2 and 3 of Paper No. 19, are traversed. Reconsideration and withdrawal of the rejections are requested in view of the following comments.

The objected-to negative limitation has been deleted from claim 22, to advance prosecution. The objected-to recitation in claims 22 and 32 is believed to be supported by the specification, for example, on pages 17 and 18 of the specification. Specifically, page 18 of the specification describes that Figures 4A and 4B refer to standard curves of the colorimetric assay for mixtures of rifampicin and plasma at several concentrations ranging from 0.39 µg/ml to 25 µg/ml. The recitations of at least 0.3 µg/ml therefore is believed to be supported by the specification. Page 17 of the specification describes that, in the case of rifampicin and its metabolites, colorimetric assays are the most preferred when simplicity and accuracy are required. The applicants submit that these discloses demonstrate that the method of the present invention is able to detect at least the noted drug to a level of 0.39 µg/ml in a colorimetric assay and the applicants submit the same has been reasonably expressed as recited in the objected-to phrase. Reconsideration and withdrawal of the Section 112, first paragraph, rejection of claims

22-36 and the "new matter" rejection recited in paragraphs 2 and 3 of Paper No. 19 is requested.

The Section 112, second paragraph, of claims 25, 30 and 32-36 stated in paragraphs 5 and 6 of Paper No. 19 is obviated by the above amendments. Reconsideration and withdrawal of the Section 112, second paragraph, rejection of claims 25, 30 and 32-36 are requested.

At a minimum, claims 32-36 are believed to be in condition for allowance and an indication of the allowability of at least claims 32-40 in the Examiner's next Action is requested. See, paragraph 18 on page 5 of Paper No. 19.

The Section 103 rejection of claims "22-, 7-10" over Meucci et al. (U.S. Patent No. 5,135,875) is traversed. See, paragraph 14 of Paper No. 19. The Examiner is urged to appreciate that claims 7-10 were canceled by way of the Amendment dated February 10, 2003. The applicants presume the Examiner has rejected claims 22-31 as allegedly being obvious over Meucci however the Examiner is requested to advise the undersigned if otherwise and provide further time to respond to any previously unstated objection or rejection of the claims in a new non-final Office Action. Reconsideration and withdrawal of the Section 103 rejection are requested in view of the following distinguishing comments.

The applicants respectfully submit that the cited patent teaches, at col.1, lines 50-65, "The present invention relates to the discovery that analytical systems for the determination of a hydrophobic analyte in a biological test sample, particularly analytical systems employing specific binding proteins for such analytes, can be substancially improved by employing the precipitation reagent of the present invention which extracts

such analyte and precipitates interfering proteins. In particular, such precipitation reagent has unexpectedly and surprisingly been found to precipitate interfering proteins, such as hemoglobin, and other interfering substances from a biological test sample while, at the same, maintaining hydrophobic analytes in solution and minimizing the denaturation of specific binding proteins, such as for example, antibodies, wich may be present in an immunoassay system."

The patent further states at col.2, lines 29 – 39 as follows:

"In particular, precipitation of interfering proteins, hemoglobin, and other intefering substances, are precipitated from the test sample to thereby render such analytes readily available for measurement by a desired analytical system. Although the precipitation reagent is particularly useful in analytical systems for determining hydrophobic analytes employing specific binding proteins, especially immunoassay systems, the precipitation reagent can be employed in other assay systems as well, such as radioassays and the like."

According to Meucci, col.2, lines 7 to 9: The precipitation reagent contains "from between about 5 percent (w/v) and about 50 percent (w/v) of a glycol".

Col. 2, lines 58 to 64 of the cited patent states that the glycol component serves to decrease the toxicity of the other components of the precipitation reagent, particularly the alcohol component, and stabilizes cellular receptors and specific binding proteins which are employed in an assay system, particularly antibodies, by preserving the binding integrity thereof .

One of ordinary skill in the art will appreciate that if the precipitation reagent of Meucci must contain a glycol component, in a minimum amount 5 percent (w/v), or it

must at least contain an equivalent functional component, i.e., a component which provides a glycol specific function. The assay systems used in Meucci employ cellular receptors and specific binding proteins for the analyte to be measured.

The "other assay systems" (col.2, lines 38-39), of the cited patent are submitted to be limited to assay systems which must use at least functionally similar reagents (i.e., cellular receptors and specific binding proteins) to detect the analyte, as the immunoassay. As one alternative to the immunoassays systems, in the paragraph containing the same col.2, lines 38-39, radioimmunoassay are described.

The Examiner recognizes that Meucci fails to explicitly teach an example using assays other than immunoassay

Consequently, the applicants submit that the expression "other assay systems" from col.2, lines 38 – 39 of Meucci cannot be considered to include or suggest, beyond immunoassays, all possible kinds of assay systems, such as those of the presently claimed invention.

The precipitation reagent of Meucci is specific. The sample preparation in Meucci is specific to assays systems employing reagents to detect the hydrophobic analytes and not to prepare other samples for any other kind of assay systems.

Meucci does not teach or suggest the use of a sample preparation method with assay systems other than using reagents to detect an analyte, in an immunoassay.

The following Table compares the "Method of monitoring patient compliance and bioavailability of drugs contained in body fluids" of the present invention with the "Protein Precipitation Reagent" of Meucci – US 5,135,875.

Meucci - US5135875 - Protein Precipitation Reagent		
Function	Means	Results
• To avoid the inconsistencies in results of conventional techniques to monitor drug levels or to detect other analytes in biological fluids. • To eliminate the disadvantages of the known precipitation reagents. Examples: - Determination of hydrophobic analytes in a whole blood test sample hemoglobin is not removed; - Loss of the binding activity of antibodies employed in an immunoassay system.	• An Immunoassay method for determining a hydrophobic analyte in a biological test sample uses the precipitation reagent to remove interfering proteins from said sample, prior to immunological detection of the analyte (claim 11). • An immunassay test kit comprising in separate containers a precipitation reagent useful for precipitating proteins and extracting a hydrophobic analyte from a biological test sample and a specific binding agen. Precipitation reagent contains: from between about 5.0 mM and about 100.00mM of a zinc salt, from between about 30% (w/v) and about 100% (w/v) of a straight or branched chain alcohol having from 1 to 4 carbon atoms, and from between about 5% (w/v) and about 50% (w/v) of a glycol. It may further contain from between about 0% (w/v) and about 20(w/v) of an acid. (col 2, lines 3 to 11). (w/v). zinc salt- zinc sulfate , zinc chloride, and zinc acetate Example: Immunoassay to determine cyclosporine in whole blood The samples of blood with cyclosporine and solubilization reagent are mixed on a vortex mixer for ten seconds and placed into a centrifuge. The fluorescence polarization value (col. 7, lines 50 to 65) of the supernatant is determined. The supernatant is combined with a detectable tracer compound and an appropriate antibody or binding agent for the analyte and measured in a fluorescence polarization immunoassay. (col.3, lines26 to 55 especially 36 to 40).	- Recovering from about 90% to 100% of the extracted analyte. (col.3, lines20-22). - The sensitivity of the preferred fluorescence polarization assay, is 15.0 nanograms / mililiter of ciclosporine and metabolites. (Example col.7, lines 18 – 20)

Present Invention - ***Method of monitoring patient compliance and bioavailability of drugs contained in body fluids***		
Function	Means	Results
• To avoid the inconsistences in results of conventional techniques to monitor drug levels in body fluids • To eliminate the disadvantages: (1) of methods with multiple extractions steps; and (2) of precipitating reagents, such as salts - ammonium sulfate, less effective in the separation of proteins from complex mixtures (blood or plasma). • To have sensitivity and specificity *sufficient* to detect traces of substances contained in *complex* body fluids. (Page 9, lines 4 to 8), • To quantify Rifampicin in order to monitor its levels in body fluids • To make tuberculosis diagnostic based on Rifampicin	o This Method is characterized by a simplified and effective deproteinizing body fluid step followed by drug extraction and measurement using an accurate technique, such as a colorimetric assay or a HPLC (High-Performance Liquid Chromatograph (page1, lines 7 to 11). o In this just one extraction step is used as prior treatment before drug level detection Aqueous zinc sulfate solution, an appropriated solvent and the body fluid, to be analyzed, are mechanically mixed, (to precipitate proteins and strip off bound drug-(page 15, lines 19-20). After centrifuging, the deproteinized supernatant phase is carefully recovered to determine drug and its metabolites concentrations (page 14, lines 1 to 7) using an accurate technique, (page15, lines 22 to 24). Precipitation reagent: 1. 0.1M to 5.0M aqueous *zinc sulfate* solution, 2. an appropriate solvent to extract the drug: (depending on the solubility properties of the drug being measured (page 15, lines 25-26 and page 16, line 1) - polar(water, an alcohol and a mixture thereof) - or non polar(acetonitrile/2- propanol, benzene, toluene, dichloromethane, ehloroform and mixtures thereof) - or mixtures thereof. 3. optionally an acid as anti-oxidizing agent Drugs: Rifampicin, an antimonial, an itraconazole, a proteinase and a reverse transcriptase inhibitor.	- Recovering at least 97% of the drug (Summary of Invention) - Drug detection in body fluids down to 0.3 µg/ml (Summary of Invention; Figures 4A and 4B-Colorimetric assay and page 18 lines 17 to 20) in a simplified and faster way.

Further, Meucci claims recite the use of a specific binding agent. Moreover, in the example of the patent, the sample, prepared in one step, is vortexed and centrifuged and the supernatant is tested to determine the fluorescence polarization value. But before the fluorescence polarization measure, the supernatant is combined with a detectable tracer compound and an appropriate antibody or binding agent for the analyte.

These data therefore reinforce the fact that in Meucci the sample is specifically prepared to measure the analyte with assays systems employing reagents to detect the drug and that Meucci differs from the instant invention that uses assay systems, which do not require reagents to detect the drug, as a colorimetric assay or a HPLC (High-Performance Liquid Chromatograph) are used.

Moreover the detection limit in the present invention was met with the colorimetric assay which is less sensitive than the fluorescence polarization of Meucci. The detection limit also depends therefore on the sensitivity of the detection technique.

Finally, the applicants note that there are differences between the precipitation reagents of the cited art and the present invention. Specifically, in the cited patent, a lower zinc salt concentration is used. Moreover, about 5% (w/v) to about 50% (w/v) of a glycol, a glycerol or a combination thereof is used in the cited patent to decrease the toxicity of the other components (alcohol), and stabilizes cellular receptors and specific binding proteins (antibodies) whereas a corresponding component is not utilized in the present invention. Further about 30% (w/v) to about 100% (w/v) of a straight or branched chain alcohol having from 1 to 4 carbon atoms is used in the cited patent to participate in maintaining the hydrophobic analyte in solution, and precipitate proteins

and conjugated proteins. In the present invention an appropriate solvent, such as, polar - H_2O, alcohols, and mixtures, nonpolar-acetonitrile/2-propanol, benzene, toluene, dichloromethane, chloroform or its mixtures, depending on the solubility properties of the drug which is being measured is used to extract the drug from the test sample. In the cited patent about 0% (w/v) to about 20% (w/v) of an acid component 5-sulfosalicylic acid, trichloroaceticacid, hydrolorric acidic acetic acid and the like is used to precipitate and denature interfering proteins. In the present invention, an antioxidant is optionally included with ascorbic acid, to slow down the occurrence of oxidation reaction.

The claims are submitted to be patentable over Meucci.

It would not have been obvious from Meucci, to have used a stronger concentration of zinc sulfate in a smaller added volume and an appropriate solvent to extract the drug (Example I) to recover at least 97% of the drug through a simplified and effective deproteinizing step from body fluids, such as in the present claim 23. As for claim 27, for example, Meucci uses acetic acid as an optional component which functions in a context different from the present invention, precipitating and denaturing interfering proteins, whereas in the present invention the acid only acts as an antioxidant.

As for claim 28, for example, it would not have been obvious to use other assay methods after the sample preparation, because Meucci teaches, as described above, that the sample is specifically prepared to measure the analyte with assays systems employing reagents to detect this analyte. In the present invention the sample preparation is different from Meucci which makes it possible to use assay methods

different than Meucci to detect the analyte in a simpler way (see Example 1) than that used in Meucci.

As for claims 29-31, for example, it would not have been obvious to use the sample preparation method on other hydrophobic drugs. The method of the present invention detects not only hydrophobic drugs and, as described above, the present invention uses a specific sample preparation method, that is different from the method employed by Meucci. The method of the present invention uses an appropriate solvent, depending on the solubility properties of the drug, which is being measured, while Meucci uses a specific sample preparation depending on the assay system to measure the drug.

Withdrawal of the Section 103 rejection is requested.

The claims are submitted to be in condition for allowance and a Notice to that effect is suggested.

Respectfully submitted,

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